Genetic transformation of human cells by a soil phytopathogen presents common molecular strategies

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Agrobacterium tumefaciens is a Gram negative, soil bacterium that causes crown gall disease in many crops. This soil phytopathogen has been extensively researched and used in plant genetic engineering. This is by virtue of the fact that *Agrobacterium* is capable of modifying the plant cell genome by the insertion of a piece of its plasmid DNA[1]. So far, genetic transformation by *Agrobacterium* is the only known natural example of trans-kingdom DNA transfer into plants. *Agrobacterium* infection requires the presence of genetic components like the transferred DNA (T-DNA) on tumour inducing (Ti) plasmid and virulence loci on Ti plasmid (*vir* genes) and on chromosome (*chv* genes). Physical contact of the bacterial cells onto wounded plant cell surface is a pre-requisite that results from the chemotactic attraction of bacterial cells toward wounded plant cells. On receiving chemical signals from the exudates of wounded plant cells like the phenolic compounds (e.g. acetosyringone), complex biochemical processes are initiated in *Agrobacterium* that result in the systematic and sequential events leading to the formation of a complex transport machinery consisting of the single stranded copy (T strand) of the T-DNA and associated proteins; targeted translocation and import of this machinery into plant cell nucleus and final illegitimate recombination resulting in the integration of the T-DNA into the plant cell genome. Once the T-DNA is inserted, several oncogenic genes and metabolite biosynthesis genes on the T-DNA involved in the neoplastic tumour growth and opine biosynthesis respectively, are expressed[2].

Therefore, plants serve as natural hosts for *Agrobacterium*. Recently, *Agrobacterium* was also shown to transfer its T-DNA to other microbial members, albeit within the kingdom, such as yeast[3], several species of filamentous fungi[4], phytopathogenic fungi[5] and a cultivated mushroom[4]. Then came an interesting report[6] from a group headed by Vitaly Citovsky of the State University of New York that *Agrobacterium* could also genetically transform human cells! These researchers used human HeLa R19 cells, human embryonic kidney (HEK) 393 cells and clonal pheochromocytoma PC12 neuronal cells along with *Petunia hybrida* cell suspension cultures as positive control. They observed the attachment of cell aggregates of *Agrobacterium* onto HeLa cells, a pre-requisite for DNA transfer. Coincubation with acetosyringone induced *Agrobacterium* resulted in genetic transformation of HeLa cells and such transformation frequency was comparable to the calcium phosphate method of stable transfection of mammalian cells (albeit at frequencies lower than that of the lipofectin transfection method). HEK393 and neuronal PC12 cells were also susceptible to genetic transformation. Southern hybridization and TAIL PCR verified *bona fide* T-DNA integration into the genome. Use of bacterial mutants defective in virulence genes that are avirulent on plant hosts failed to transform HeLa cells implicating that *Agrobacterium* likely uses its plant transformation protein machinery for transformation of HeLa cells also.

Recently, in the same lab, the events leading to T-DNA uptake by HeLa cells and its integration into genome were further investigated. Roles of bacterial (VirE2), plant (VIP1) and HeLa cell (Karyopherin α) proteins including their specific interactions have been identified, in this trans-kingdom T-DNA transfer story[7].

These experiments have been conducted under lab conditions. However, the biological relevance of DNA transfer from *Agrobacterium* to human HeLa cells in nature is at present far from evident. *Agrobacterium* and humans as such, represent evolutionarily two extreme cases across which T-DNA transfer can take place. *Agrobacterium* has a point or two, as a phytopathogen, to infect plants and transfer its DNA naturally. The T-DNA contains genes that cause hyperplastic tumours and also genes for opine biosynthesis which *Agrobacterium* uses as source of food, all without invoking hypersensitive response in the hosts unlike most other pathogens. This ingenuous strategy of genetically modifying the host, making host cells as hostages and subverting host components for its benefit makes *Agrobacterium* unique and a typical reference point even among medical pathologists and researchers working in the area of bacterial conjugation and macromolecular transfer (specifically Type 4 secretion) systems[8].

But why HeLa cells? Are there any human bacterial pathogens that perform similar tricks of transferring DNA or effector proteins across kingdoms into human cells using the Type 4 secretion system? How are they related to soil phytopathogenic *Agrobacterium*? Do these bacteria share a common pathogenicity strategy? Recent studies indicate a positive answer to some of these and other questions that are equally fascinating to plant biologists and are troubling medical pathologists. A reference case is *Bartonella henselae*, the causal agent of cat scratch disease. Both *Agrobacterium* and *Bartonella* belong to the α-2 subgroup of proteobacteria. *Bartonella* causes vasculoproliferative disorders including bacillary angiomatosis (cutaneous and mucocutaneous localizations) and bacillary peliosis of internal organs (e.g. peliosis hepatis) in immunocompromised individuals. A recent report indicates that *Bartonella* also causes Leber's idiopathic stellate neuroretinitis (optic neuritis)[9]. The similarities in the group and *modus operandi* between *Agrobacterium* and *Bartonella* are indeed very striking: 1, Both belong to the same (α-2) subgroup of proteobacteria; 2, Both share ~ 95% similarity in their 16S rRNA sequence; 3, Both harbour a highly homologous Type 4 secretion system gene cluster (the *virB* operon)[10]; 4, Both have pili mediating attachment to host cells; 5, Both possess hormonal factors (auxins and cytokinins in *Agrobacterium* and vascular endothelial growth factor and interleukin in *Bartonella*) of tumour growth; 6, Both inhibit pathogen-induced programmed cell death of their hosts and 7, Both share common molecular events operating tumour formation, cell hostage and sensing mechanisms. As if these are not sufficient, even by analogy, human tumours and plant tumours reveal striking organizational similarities[11,12]!

Table 1. Trans-kingdom transfer of macromolecular pathogenicity structures

Pathogen	Disease	Macromolecule transferred
Agrobacterium	Crown gall	T-DNA
Bartonella henselae	Cat scratch	VirB protein
Helicobacter pylori	Mucosa associated lymphatic tissue-lymphoma Gastric adenocarcinoma Peptic ulcer	CagA protein
Bordetella pertussis	Whooping cough	Ptl protein
Listeria monocytogenes	Listeriosis	Invasin???
Yersinia enterocolitica	Yersiniosis	Invasin???
Brucella suis	Brucellosis	VirB protein

Trans-kingdom transfer of effector proteins from bacteria to plants and animals including humans through the Type 4 secretion system may not be uncommon. There are a number of other human pathogenic microbes that do such transfers (Table 1)[11,13]. But what is more striking is that the soil phytopathogen, *Agrobacterium*, is the only one example wherein trans-kingdom (T-)DNA transfer takes place in plants (and now in humans too) using the same Type 4 secretion system. Also, *Agrobacterium* represents the best characterized member in the group in terms of Type 4 secretion system (and, of course, best utilized for plant genetic engineering and transgenosis)[14]. The successful research attempts to make human cells also susceptible to *Agrobacterium* infection and T-DNA transfer opens up new interesting areas coupled with fresh batches of questions that demand greater validations and interpretations. Similarly, already there are expressed apprehensions by a few anti-genetic engineering groups, globally, regarding the biosafety concerns of using *Agrobacterium*. As per the available information, these concerns are misplaced as there are no reports to indicate that humans (as opposed to cell lines) are transformable by *Agrobacterium*.

The research findings of Citovsky's group that only a few proteins may be necessary for human cells to uptake specific foreign DNA coupled with the ever-burgeoning information on *Agrobacterium* molecular biology, general biology of the Type 4 secretion system and molecular relatedness of *Agrobacterium* and other human pathogens including *Bartonella* – are all leading to a very exciting period. New breakthroughs can be anticipated in our understanding of the role of the Type 4 secretion system in microbial pathogenesis and its application in the genetic transformation of plant and human cells.

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COMMENTARY

HIV/AIDS in the developing world*

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During the past two decades, HIV/AIDS has had a devastating impact on the health and social and economic well-being of populations in many parts of the developing world.

In 2003 alone, HIV/AIDS caused the death of more than three million people (All figures cited are based on UNAIDS, WHO and CDC data.). That made it the number-one killer among all infectious diseases.

The vast majority of the 40 million people living with HIV/AIDS – indeed some 34 million or nearly 85% of the total number of people afflicted with the disease – are in Africa, Asia and Latin America, among countries that are least able to manage the epidemic or afford the costly combination of antiretroviral drugs which have dramatically reduced AIDS-related morbidity and mortality rates in developed countries.

Sub-Saharan Africa, on its own, accounts for about two-thirds of all HIV/AIDS-related deaths. The region also accounts

*Joint Statement by Third World Academy of Sciences and African Academy of Sciences, Trieste, Italy/Nairobi, Kenya, July 2004.